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Phenolic composition and antioxidant properties of *ex-situ* conserved tomato (*Solanum lycopersicum* L.) germplasm

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) local varieties represent a reservoir of genetic diversity for desirable quality traits. In this study, a representative collection of table tomato germplasm conserved *ex-situ* in the Portuguese Gene Bank was characterized for its polyphenols composition and antioxidant capacity. Phenolic acids, such as caffeic and *p*-coumaric acids bounded to a hexose and 5-*O*-caffeoylquinic acid, corresponded to 71–98% of the identified phenolic compounds; while the remaining fraction consisted of quercetin and kaempferol glycoside derivatives. Among the studied tomato accessions, it was possible to identify those that stand out for the analysed bioactive traits. These findings highlighted the interest of using Portuguese tomato germplasm in breeding programs or of reintroducing into cultivation these local varieties used for fresh consumption.

1. Introduction

Tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum* Mill.) is an important component of the human diet worldwide and a rich source of micronutrients and natural antioxidants, including carotenoids (mainly lycopene), ascorbic acid, potassium, folate and phenolic compounds (Barros et al., 2012; Pinela, Barros, Carvalho, & Ferreira, 2012). Although the phenolic content in tomatoes and tomato-based products is not outstanding, the high consumption of these foods (~35 kg *per capita* in 2013 in Southern Europe; FAOSTAT (2017)) makes them surpass several other fruits and vegetables with higher contents of these health-promoting compounds (George, Kaur, Khurdiya, & Kapoor, 2004; Pinela, Oliveira, & Ferreira, 2016).

Phenolic compounds have been extensively characterized in diverse tomato genotypes. Chlorogenic acids and related compounds (hydroxycinnamates) have been reported as the main phenolic compounds besides flavonoids (such as rutin, quercetin, naringenin, chalconaringenin and kaempferol derivatives) (Barros et al., 2012; Martínez-Valverde, Periago, Provan, & Chesson, 2002; Siracusa, Patanè, Rizzo, Cosentino, & Ruberto, 2018; Slimestad, Fossen, & Verheul, 2008; Slimestad & Verheul, 2009). Among these secondary metabolites, chlorogenic acid is an important and biologically active dietary polyphenol, which plays several therapeutic roles as antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, hypoglycemic, antimicrobial, and antiviral agent (Naveed et al., 2018). In turn, quercetin exhibits significant heart related benefits and anti-inflammatory, anti-aggregant, and vasodilating effects *in vivo* (Patel et al., 2018); and rutin, also known as vitamin P, has anticarcinogenic effect and ability to reduce the fragility of blood vessels (Pandey et al., 2018). These polyphenols may also be related to a somewhat tomato astringency (Gómez-López, 2012). However, the chemical composition of this fruit can vary according to the variety or cultivar, cultivation technique, handling, and storage conditions (Barros et al., 2012; Di Paola Naranjo et al., 2016; Georgé et al., 2011; Liu, Zheng, Sheng, Liu, & Zheng, 2018; Pinela et al., 2012; Siracusa et al., 2018; Slimestad et al., 2008). In fact, phenolic compounds can be used as cultivar-distinguishing factors in some plant foods (Klepacka, Gujska, & Michalak, 2011).

The growing consumer demand for premium quality tomato varieties has promoted the adoption of breeding strategies to increase the accumulation of antioxidant and health-promoting compounds in this highly consumed fruit (Bertin & Génard, 2018; Tohge, Alseekh, & Fernie, 2014). Generally, the commercial tomato varieties do not contain high amounts of phenolic compounds, which in part may be due to the loss of genetic and chemical diversity caused by the domestication

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process of this crop (Kamenetzky et al., 2010; Perez-Fons et al., 2015; Tohge et al., 2015). In addition, most of the modern varieties contain the *uniform ripening* (*u*) gene that inhibits the synthesis of sugars and aroma compounds (Powell et al., 2012). Therefore, one of the strategies currently used to improve the healthy profile of tomatoes considers reintroducing and screening wild genetic resources, old farmer varieties, landraces, and gene bank accessions for quality traits (such as polyphenols content) that could be introduced into modern varieties or cultivars (Csambalik et al., 2017; Martínez-Vázquez et al., 2017; Rigano et al., 2016; Siracusa et al., 2018).

Following this strategy, this study was performed to identify and select polyphenol-rich tomato germplasm with potential to be used in breeding programs. Therefore, 18 accessions of a representative collection of table tomato local varieties conserved *ex-situ* in the Portuguese Gene Bank (BPGV) was first regenerated in experimental fields and the phenolic profile of samples from the obtained plant populations of each accession were then characterized by HPLC-DAS-ESI/MS and the antioxidant capacity evaluated using different *in vitro* assays.

2. Materials and methods

2.1. Plant material

Plant materials of this study concern the ripe fruits (*e.g.* > 90% of the pericarp surface coloured) of table tomato local varieties selected among the germplasm collection of the Portuguese Gene Bank (BPGV). BPGV large seed collection of cultivated species results from systematic and coordinated efforts for *ex-situ* conservation of plant genetic resources, particularly regional and farmer varieties linked with traditional agricultural systems and local knowledge. Since 1970, the BPGV has conducted several national and international collecting missions in all Portuguese territory to gather crop diversity ensuring sustainable strategic objectives.

Therefore, 18 accessions (common term corresponding to an individual sample in a gene bank, such as a distinct species or variety) of Portuguese tomato local varieties from the BPGV collection, were selected for germplasm regeneration (replenishing seed stocks) and characterization. Germplasm characterization describes plant germplasm, providing information about highly heritable characters ranging from morphological or agronomical features to chemical and molecular traits, assuring the maximum utilization of the germplasm collection to the final users. These processes follow international guidelines and standards for plant germplasm conservation *e.g.* gene bank standards for plant genetic resources (FAO, 2014) and FAO/Bioversity Multi-crop passport descriptors (Alercia, Diulgheroff, & Mackay, 2015). The passport code, local name and geographic origin and main features of the studied accessions are described in Table 1, as well as basic information about their fruits. These local varieties are regionally named and known by a vernacular name, which might be related with the growth habit of the plant, the size and usual shape of the fruit, or with some other locally valuable unique features, such as pulp texture, mode of consumption or organoleptic characteristics. For instance, "coração-de-boi" (meaning ox-heart) tomatoes are usually large fruits with a meaty texture.

Since we used plant materials from a regeneration germplasm task, usually performed in crop seed banks, the seeds of the selected accessions (18 accessions) were cultivated on BPGV experimental fields in Braga, Portugal, according to FAO/IPGRI Genebank Standards (2014). Harvesting was done at optimum maturity of seeds (after the seeds have reached the point of physiological maturity), which was determined by visual methods at the full maturity stage of fruits defined using the criteria of IBPGR (1996). The geographical and edaphoclimatic characteristics of the plantation local are described in Supplementary Table 1. All studied accesses were installed under the same soil and climatic conditions and agronomic management (*e.g.*, irrigation, pruning, phytosanitary treatments, harvesting techniques). Each accession was grown under spatial isolation by artificial barriers, *e.g.* large box made of net. Therefore, it is expected that this approach reduced abiotic effects on the chemical composition of the analysed plant material.

The studied ripe fruits of each accession were randomly hand harvested within the respective population, transported to the laboratory where they were frozen at −20 °C to be lyophilized (FreeZone 4.5 model 7,750,031, Labconco, Kansas City, USA) and then reduced to a fine dried powder (~20 meshes) that was kept at −20 °C until analysis.

2.2. Preparation of hydroethanolic extracts

Tomato hydroethanolic extracts were prepared according to a procedure previously described by Barros, Carvalho, and Ferreira (2010), with some modifications. Briefly, the powdered samples (~1 g) were stirred with ethanol/water (80:20, *v/v*; 30 mL) for 1 h at room temperature. The supernatant was filtered through Whatman No. 4 filter paper and the residue was re-extracted with an additional portion of solvent (30 mL) under the same conditions. The ethanolic fraction of the filtrates was then removed at 40 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and the remaining aqueous phase was lyophilised. Three dried extracts from each sample were prepared and analysed for phenolic composition and antioxidant capacity.

2.3. HPLC-DAD-ESI/MS^n analysis of phenolic compounds

The extracts were purified using Sep-Pak C18 3 cc Vac Cartridges (Phenomenex, Torrance, CA, USA), activated with methanol followed by water; the samples (at ~20 mg/mL in water; 10 mL) were eluted through the cartridge and sugars and other polar substances were removed by passing 15 mL of water. The phenolic compounds were further eluted with methanol (5 mL). Thereafter, the purified extracts were concentrated under reduced pressure, redissolved in methanol/water (20:80, *v/v*; 2 mL), and filtered through 0.22-μm disposable LC filter disks.

The analysis was performed in triplicate by HPLC-DAD-ESI/MS^n (Dionex Ultimate 3000 UHPLC, Thermo Scientific, San Jose, CA, USA) as previously described (Bessada, Barreira, Barros, Ferreira, & Oliveira, 2016). A Waters Spherisorb S3 ODS-2 C18 column (3 μm, 4.6 mm × 150 mm; Waters, Milford, MA, USA) was used for chromatographic separation. Double online detection was carried out using DAD (using 280 and 370 nm as preferred wavelengths) and a mass spectrometer (MS). MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Phenolic compounds were identified by comparing their retention times, UV–vis and mass spectra with those obtained from standard compounds, when available; otherwise, compounds were tentatively identified comparing the obtained information with available data reported in the literature.

For quantitative analysis, a calibration curve for each available phenolic compound standard (from Extrasynthèse, Genay, France) was constructed based on the UV signal (A: caffeic acid, $y = 388{,}345\times + 406{,}369$, $R^2 = 0.994$; B: *p*-coumaric acid, $y = 301{,}950\times + 6966.7$, $R^2 = 0.999$; C: chlorogenic acid, $y = 168{,}823\times - 161{,}172$, $R^2 = 0.999$; D: syringic acid, $y = 376{,}056\times + 141{,}329$, $R^2 = 0.9995$; E: quercetin-3-*O*-rutinoside, $y = 13{,}343\times - 76{,}751$, $R^2 = 0.9998$; and F: kaempferol-3-*O*-rutinoside, $y = 11{,}117\times + 30{,}861$, $R^2 = 0.999$; other analytical quality parameters are given in Supplementary Table 2). Quantification of the phenolic compounds that are not commercially available as standards was performed by assuming that their molar absorptivity is the same as that of the corresponding free standard molecule (see Supplementary Table 2). The results were expressed as μg per g of extract.

Table 1
Local name, basic description and geographical origin of the Portuguese table tomato accessions *ex-situ* conserved in the BPGV.

Tomato accessions main features						Basic description of the ripe fruit				Geographic origin	
Accession number	PT local name (literal meaning in EN)	Similar commercial type	Growth habit	Fruiting period (days)	Fruits per plant	Shape	Mean weight (g)	Number of locules	Size	Region	Collecting site
BPGV 11098	Tomate maçã (apple tomato)	Flattened tomato	Indeterminate	80	8	Globe indented	504	11	Large	Lisbon	Manique do Intendente, Azambuja
BPGV 11350	Tomate de cacho, liso e redondo (round and smooth fruits in vines)	Round standard	Indeterminate	84	19	Round indented	143	5	Medium	Lisbon	Manique do Intendente, Azambuja
BPGV 11363	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	88	20	Heart-shape	325	11	Large	Santarém	Arrouquelas, Rio Maior
BPGV 11372	Tomate	Round standard	Indeterminate	86	10	Heart shape	344	11	Large	Leiria	Arrimal, Porto de Mós
BPGV 11400	Tomate maçã (apple)	Flattened tomato	Determinate	84	10	Round	154	6	Medium	Santarém	Amiais de Baixo, Santarém
BPGV 11465	Tomate vermelho (red)	Round standard	Determinate	76	11	Round indented	218	8	Large	Santarém	Carvoeiro, Mação
BPGV 11681	Tomate	Round standard	Semi determinate	68	3	Round	165	3	Medium	Santarém	Bemposta, Abrantes
BPGV 11696	Tomate redondo (round tomato)	Round standard	Semi determinate	77	13	Round	138	5	Medium	Santarém	São José da Lamarosa, Coruche
BPGV 11803	Tomate grosso (thick fruit tomato)	Round standard	Semi determinate	67	11	Flat-round	194	7	Medium	Portalegre	Santa Maria de Marvão
BPGV 11907	Tomate cabecinhas (small heads tomato)	Plum tomato	Determinate	69	17	Ellipsoid	92	2	Small	Portalegre	Aldeia Velha, Avis
BPGV 12260	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Determinate	83	12	Flat-round	189	2	Medium	Bragança	Santulhão, Vimioso
BPGV 12437	Tomate amarelo (yellow tomato)	Yellow tomato	Determinate	64	9	Deep oblate	208	5	Large	Bragança	Águas Vivas, Miranda do Douro
BPGV 12465	Tomate sem varas (no staking tomato)	Round standard	Determinate	64	8	Round	129	4	Medium	Bragança	Peredo da Bemposta, Mogadouro
BPGV 12506	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Semi determinate	81	13	Heart shape	260	10	Large	Santarém	Santarém
BPGV 12906	Tomate	Elongated Roma	Indeterminate	76	16	Cylindrical	188	3	Medium	Aveiro	Frossos, Albergaria-a-Velha
BPGV 12954	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	79	17	Heart shape	271	9	Large	Aveiro	Válega, Ovar
BPGV 13034	Tomate coração-de-boi (oxheart tomato)	Beefsteak tomato	Indeterminate	83	13	Heart shape	411	15	Large	Guarda	Teixeira, Seia
BPGV 16388	Tomate salada (salad tomato)	Roma tomato	Determinate	83	41	Cylindrical	126	2	Medium	Castelo Branco	Proença-a-Nova

Basic description of the ripe fruit was performed in 12 plants from the population of each accession. Data within Table 1 correspond to the mean values for each accession. Fruit size: Large > 200 g; Medium 100–200 g; small < 100 g.

Table 2
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and tentative identification of phenolic compounds in the Portuguese table tomato accessions described in Table 1.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 fragments (*m/z*)[a]	Tentative identification
1	5.0	320	341	179(100)	Caffeic acid hexoside I
2	5.3	306	325	163(100)	*p*-Coumaric acid hexoside
3	6.1	325	353	191(11), 179(47), 173(100), 135(5)	4-*O*-Caffeoylquinic acid
4	6.3	320	341	179(100)	Caffeic acid hexoside II
5	6.8	326	353	191(100), 179(11), 161(15), 135(6)	5-*O*-Caffeoylquinic acid
6	7.0	314	325	163(100)	*p*-Coumaric acid hexoside
7	7.3	312	325	163(100)	*p*-Coumaric acid hexoside
8	8.2	320	341	179(100)	Caffeic acid hexoside III
9	9.3	320	179	135(100)	Caffeic acid
10	13.5	274	359	197(100), 153(35), 135(5)	Syringic acid hexoside
11	14.4	309	163	119(100)	*p*-Coumaric acid
12	15.0	350	741	609(47), 301(100)	Quercetin-pentosyl-rutinoside
13	17.0	352	609	301(100)	Quercetin-3-*O*-rutinoside (rutin)
14	20.1	348	593	285(100)	Kaempferol-3-*O*-rutinoside
15	23.6	292, 311	917	741(100), 609(44), 301(31)	Quercetin-*O*-feruloyl-pentosyl-*O*-rutinoside

[a] Figures in brackets after MS^2 fragment ions refer to their relative abundances.

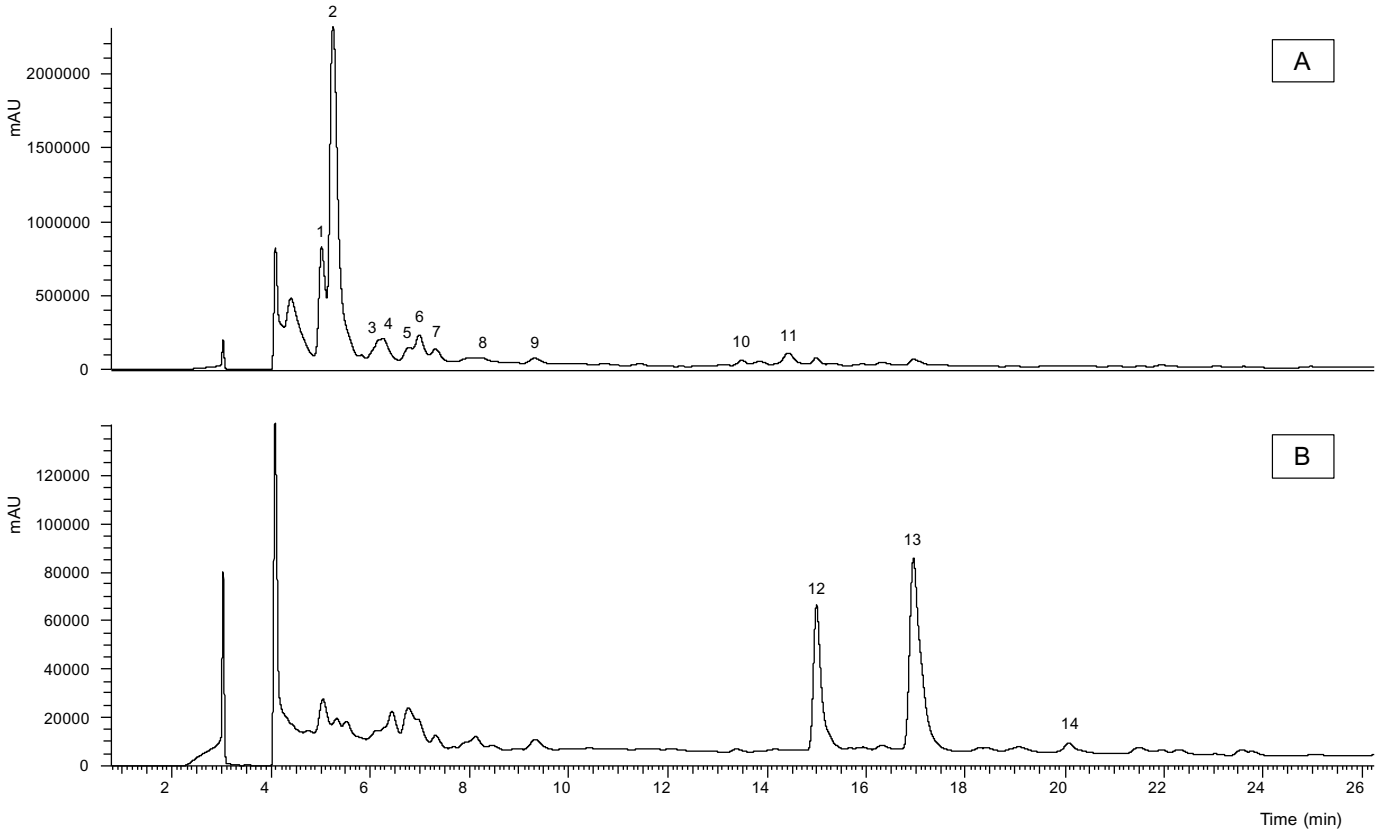

Fig. 1. HPLC phenolic profile of table tomato accession BPGV 11803 recorded at (A) 280 nm and (B) 370 nm. See Table 2 for peak identification.

2.4. Evaluation of the antioxidant capacity

The extracts were redissolved in ethanol/water (80:20, *v/v*) to obtain a 50 mg/mL stock solution, from which extract solutions with different concentrations (10–0.08 mg/mL) were prepared by successive dilutions. The antioxidant capacity of these extracts was then evaluated in triplicate following *in vitro* assays previously described by Pinela et al. (2012): i) β-carotene bleaching inhibition capacity in the presence of linoleic acid free radicals; ii) thiobarbituric acid reactive substances (TBARS) formation inhibition capacity using porcine brain cells as models; iii) DPPH free radical-scavenging activity; and iv) reducing power (ferricyanide/Prussian blue assay). Trolox was used as positive control. The results were expressed in EC_{50} values (mg/mL), *i.e.*, extract concentration providing 50% of antioxidant capacity or 0.5 of absorbance in the reducing power assay.

2.5. Statistical analysis

All extractions were performed in triplicate and each replicate was analysed three times ($n = 9$). Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS Statistics software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

The fulfilment of the one-way analysis of variance (ANOVA) requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and

Table 3
Content (μg/g of extract)[a] of phenolic compounds in the Portuguese table tomato accessions described in Table 1. See Table 2 for peaks identification.

Peak[b]	BPGV11098	BPGV11350	BPGV11363	BPGV11372	BPGV11400	BPGV11465	BPGV11681	BPGV11696	BPGV11803
1[A]	206 ± 4 [c]	100.2 ± 0.3 [j]	174 ± 2 [e]	140 ± 4 [g]	53.3 ± 0.-2[a]	154 ± 5 [f]	58 ± 2[k,a]	196 ± 1 [d]	246 ± 2 [b]
2[B]	867 ± 19 [c]	170 ± 2 [m]	524 ± 10 [g]	667 ± 10 [e]	193 ± 3[a,-m]	936 ± 5 [b]	253 ± 4 [j]	681 ± 2 [e]	1250 ± 32 [a]
3[C]	152 ± 4 [d,e,f]	140 ± 1 [e,f]	184 ± 1 [b,c]	81.7 ± 0.4 [j,k]	79 ± 3 [k]	122 ± 9 [g]	94 ± 2 [i,j]	116 ± 10 [g,h]	187 ± 11 [b,c]
4[A]	114 ± 1 [b]	73.9 ± 0.9 [e]	65 ± 2 [f]	87 ± 3 [d]	46.8 ± 0.5 [h]	75 ± 1 [e]	75 ± 2 [e]	85 ± 2 [d]	61 ± 4 [f,g]
5[C]	136 ± 2 [h]	nd	133 ± 1 [h]	79 ± 2[a]	343 ± 3 [b]	117 ± 4 [i]	262 ± 6 [c]	216 ± 3 [d]	160 ± 3 [f]
6[B]	122 ± 4 [c]	128 ± 3 [b]	tr	92 ± 2 [h]	44 ± 2 [j,k]	106 ± 1 [f]	59.6 ± 0.4 [i]	88 ± 2 [h]	114 ± 2 [d,e]
7[B]	nd	nd	nd	nd	nd	nd	nd	nd	85.2 ± 05 [b]
8[A]	nd	66 ± 2 e	nd	65 ± 2 [e]	80 ± 4 [d]	nd	112 ± 4 [a]	48.3 ± 0.9 [f]	tr
9[A]	50.7 ± 0.6 [d]	nd	50.3 ± 0.5 [d]	tr	44 ± 1 [e]	nd	tr	36 ± 1 [f]	41.6 ± 0.2 [e]
10[D]	55.6 ± 0.6 [b]	43.9 ± 0.5 [d]	24.6 ± 0.7 [j]	32.6 ± 0.4 [g]	43.5 ± 0.5 [d]	32 ± 1 [g]	41 ± 1 [e]	29 ± 1 [i]	30.4 ± 0.5 [h]
11[B]	144 ± 6 [c]	152 ± 3 [a]	86 ± 2 [f]	150 ± 1 [a,b]	nd	119 ± 3 [d]	tr	38.7 ± 0.7 [i]	96 ± 2 [e]
12[E]	21.9 ± 0.3 [k]	59.2 ± 0.4 [f]	21.0 ± 0.2 [k,a]	19.9 ± 0.5[a,m]	58.2 ± 0.5 [f]	tr	48 ± 1 [h]	50 ± 2 [g]	tr
13[E]	301 ± 3 [a]	245 ± 4 [d]	29.6 ± 0.7 [n]	119 ± 1 [j]	135 ± 1 [h,i]	71.2 ± 0.7 [k]	141 ± 2 [h]	249 ± 6 [c,d]	57.4 ± 0.9[a]
14[F]	42.1 ± 0.6 [a]	17.3 ± 0.2 [f]	nd	14.4 ± 0.6 [h]	tr	tr	tr	tr	tr
15[E]	nd	nd	nd	nd	nd	nd	nd	nd	nd
ΣPA	1847 ± 29 [c]	874 ± 2 [l]	1240 ± 10 [i]	1395 ± 18 [h]	927 ± 5 [k]	1662 ± 30 [e]	955 ± 10 [k]	1534 ± 22 [g]	2272 ± 24 [b]
ΣFL	365 ± 4 [a]	322 ± 4 [b]	50.6 ± 0.7 [j]	153 ± 1 [h]	194 ± 1 [g]	71 ± 1 [i]	189 ± 3 [g]	300 ± 8 [c]	57 ± 1 [j]
ΣFC	2212 ± 33 [c]	1196 ± 7 [j,k]	1291 ± 11 [i]	1548 ± 17 [h]	1120 ± 4[a]	1733 ± 30 [f]	1144 ± 13 [k,a]	1834 ± 30 [e]	2329 ± 23 [b]

Peak[b]	BPGV11907	BPGV12260	BPGV12437	BPGV12465	BPGV12506	BPGV12906	BPGV12954	BPGV13034	BPGV16388	H[c]
1[A]	53 ± 2 [k,a]	118 ± 2 [h]	85.8 ± 0.9 [j]	146 ± 5 [g]	155 ± 3 [f]	61 ± 2 [k]	240 ± 3 [b]	106 ± 4 [i]	464 ± 10 [a]	0.097
2[B]	213 ± 3 [k,a]	451 ± 12 [h]	103 ± 3 [n]	675 ± 3 [e]	724 ± 3 [d]	237 ± 8 [j,k]	562 ± 4 [f]	317 ± 15 [i]	1260 ± 15 [a]	0.042
3[C]	118 ± 5 [g,h]	153 ± 6 [d,e]	138 ± 1 [e,f]	77 ± 5 [k]	158 ± 9 [d]	104 ± 4 [h,i]	196 ± 5 [b]	179 ± 9 [c]	314 ± 4 [a]	0.131
4[A]	39 ± 2 [i]	56 ± 2 [g]	37.4 ± 0.2 [i]	108 ± 1 [b,c]	61 ± 3 [f,g]	56 ± 1 [g]	104 ± 4 [c]	47 ± 2 [h]	352 ± 8 [a]	0.106
5[C]	nd	91 ± 2 [k]	92.5 ± 0.1 [k]	146 ± 3 [g]	112 ± 1 [i]	103 ± 4 [j]	200 ± 1 [e]	113 ± 1 [i]	376 ± 6 [a]	0.090
6[B]	61.7 ± 0.4 [i]	41 ± 1 [k]	33 ± 1 [l]	101 ± 2 [g]	115 ± 4 [d]	46.7 ± 0.3 [j]	110 ± 1 [e,f]	34 ± 2[a]	219 ± 4 [a]	0.206
7[B]	nd	24.9 ± 0.9 [d,e]	22.3 ± 0.7 [e]	nd	80 ± 3 [c]	28.5 ± 0.2 [d]	nd	26 ± 1 [d]	231 ± 7 [a]	0.001
8[A]	88 ± 4 [c]	20 ± 1 [h]	20.1 ± 0.7 [h]	nd	107 ± 2 [b]	30 ± 2 [g]	80 ± 1 [d]	31.6 ± 0.2 [g]	tr	0.025
9[A]	28.4 ± 0.7 [h]	32.2 ± 0.9 [g]	18.1 ± 0.5 [j]	49.7 ± 0.7 [d]	74 ± 1 [b]	31.6 ± 0.5 [g]	54 ± 1 [c]	23.5 ± 0.5 [i]	153 ± 5 [a]	0.005
10[D]	43 ± 1 [d]	13.9 ± 0.3 [a]	22.9 ± 0.2 [k]	nd	52 ± 1 [c]	23.0 ± 0.7 [k]	38.9 ± 0.4 [f]	21.6 ± 0.5 [k]	132 ± 1 [a]	0.515
11[B]	41.5 ± 0.1 [i]	51 ± 1 [h]	30 ± 2 [j]	65 ± 2 [g]	146 ± 1 [b,c]	67.8 ± 0.3 [g]	tr	55 ± 2 [h]	118 ± 3 [d]	0.039
12[E]	62 ± 2 [e]	92 ± 1 [b]	100 ± 1 [a]	39 ± 1 [i]	tr	68.2 ± 0.2 [d]	19.0 ± 0.4 [m]	87.2 ± 0.3 [c]	32.0 ± 0.3 [j]	0.027
13[E]	186 ± 1 [e]	176 ± 1 [f]	130 ± 2 [i]	245 ± 4 [d]	130 ± 4 [i]	172 ± 1 [f]	269 ± 3 b	163 ± 8 [g]	255 ± 8 [c]	0.083
14[F]	tr	14.6 ± 0.8 [h]	19.1 ± 0.4 [e]	tr	25 ± 2 [c]	21.7 ± 0.5 [d]	11.1 ± 0.1 [i]	16.0 ± 0.4 [g]	28.7 ± 0.6 [b]	0.007
15[E]	nd	tr	tr	nd	tr	tr	tr	tr	tr	–
ΣPA	686 ± 14 [n]	1053 ± 6 [j]	602 ± 4 [o]	1367 ± 21 [h]	1786 ± 28 [d]	788 ± 14 [m]	1586 ± 15 [f]	955 ± 22 [k]	3619 ± 53 [a]	0.263
ΣFL	248 ± 2 [f]	283 ± 1 [d]	250 ± 1 [f]	284 ± 5 [d]	155 ± 3 [h]	262 ± 1 [e]	299 ± 3 [c]	267 ± 8 [e]	315 ± 9 [b]	0.056
ΣFC	934 ± 14 [n]	1336 ± 6 [i]	852 ± 5 [o]	1651 ± 26 [g]	1941 ± 25 [d]	1050 ± 15 [m]	1885 ± 18 [e]	1221 ± 29 [j]	3935 ± 62 [a]	0.239

ΣPA: sum of phenolic acids; ΣFL: sum of flavonoids; ΣPC: sum of phenolic compounds; nd: not detected; tr: traces. All superscript letters next to the mean ± SD values refer to the statistical analysis.

[a] The results are presented as mean ± standard deviation.

[b] The superscript letters in each peak number correspond to the standard phenolic compound used for quantification (see Supplementary Table 2).

[c] Homoscedasticity (H) was tested by means of the Levene's test: $p > .05$ indicates homoscedasticity (statistical differences classified by a Tukey's HSD test), and $p < .05$ indicates heteroscedasticity (statistical differences classified by a Tamhane's T2 test). A one-way ANOVA showed that, in each line, the mean value of the evaluated parameter of at least one tomato accession differed from the others ($p < .001$ in all cases); the different letters represent significant differences.

Table 4
Antioxidant capacity (EC_{50} values, mg/mL)[a] of the hydroethanolic extracts prepared from the Portuguese table tomato accessions described in Table 1.

	β-Carotene bleaching inhibition	TBARS formation inhibition	DPPH• scavenging activity	Reducing power
BPGV 11098	0.47 ± 0.01 [c]	1.20 ± 0.07 [g]	7.21 ± 0.06 [f]	0.92 ± 0.01 [j]
BPGV 11350	0.51 ± 0.03 [b]	2.34 ± 0.08 [c]	7.05 ± 0.07 [f,g]	1.69 ± 0.04 [f]
BPGV 11363	0.39 ± 0.01 [e,f]	2.2 ± 0.1 [d,e]	6.89 ± 0.02 [g,h]	1.91 ± 0.01 [e]
BPGV 11372	0.31 ± 0.01 [h,i]	2.26 ± 0.07 [c,d]	9.8 ± 0.1 [a]	2.15 ± 0.08 [d]
BPGV 11400	0.59 ± 0.01 [a]	2.7 ± 0.1 [a]	5.85 ± 0.09 [i]	1.47 ± 0.04 [g]
BPGV 11465	0.479 ± 0.003 [c]	1.24 ± 0.09 [g]	8.43 ± 0.02 [d]	0.54 ± 0.01 [m]
BPGV 11681	0.36 ± 0.01 [g]	2.11 ± 0.06 [e]	8.7 ± 0.2 [c]	0.73 ± 0.01 [l]
BPGV 11696	0.48 ± 0.02 [c]	2.5 ± 0.1 [b]	7.23 ± 0.05 [f]	1.04 ± 0.03 [i]
BPGV 11803	0.53 ± 0.03 [b]	1.03 ± 0.03 [h,i]	9.19 ± 0.12 [b]	1.06 ± 0.04 [i]
BPGV 11907	0.51 ± 0.02 [b]	1.13 ± 0.03 [g,h]	7.96 ± 0.09 [e]	0.86 ± 0.02 [k]
BPGV 12260	0.319 ± 0.007 [h]	0.66 ± 0.04 [k]	5.05 ± 009 k	3.66 ± 0.01 [a]
BPGV 12437	0.26 ± 0.01 [j]	0.96 ± 0.01 [i]	3.70 ± 0.03 [l]	3.01 ± 0.04 [b]
BPGV 12465	0.430 ± 0.004 [d]	1.779 ± 0.004 [f]	9.11 ± 0.09 [b]	1.28 ± 0.01 [h]
BPGV 12506	0.286 ± 0.004 [i]	2.11 ± 0.02 [e]	5.6 ± 0.2 [j]	0.85 ± 0.01 [k]
BPGV 12906	0.38 ± 0.02 [f,g]	0.66 ± 0.05 [k]	6.7 ± 0.4 [h]	3.04 ± 0.03 [b]
BPGV 12954	0.41 ± 0.02 [d,e]	0.45 ± 0.01 [l]	5.41 ± 0.09 [j]	0.92 ± 0.02 [j]
BPGV 13034	0.30 ± 0.01 [h,i]	0.81 ± 0.06 [j]	6.0 ± 01 [i]	2.94 ± 0.01 [c]
BPGV 16388	0.479 ± 0.002 [c]	1.14 ± 0.04 [g]	7.07 ± 0.06 [f,g]	0.77 ± 0.03 [l]
Homoscedasticity[b]	0.000	0.000	0.000	0.000

Trolox EC_{50} values: 41 ± 1 μg/mL (reducing power), 42 ± 1 μg/mL (DPPH• scavenging activity), 18 ± 1 μg/mL (β-carotene bleaching inhibition) and 23 ± 1 μg/mL (TBARS formation inhibition).

[a] The results are presented as mean ± standard deviation. The higher EC_{50} values correspond to a lower antioxidant activity.

[b] Homoscedasticity was tested by means of the Levene's test: $p > .05$ indicates homoscedasticity (statistical differences classified by a Tukey's HSD test), and $p < .05$ indicates heteroscedasticity (statistical differences classified by a Tamhane's T2 test). A one-way ANOVA showed that, in each column, the mean value of the evaluated parameter of at least one tomato accession differed from the others ($p < .001$ in all cases); the different letters represent significant differences.

Levene's tests, respectively. Depending on the homoscedasticity, the dependent variables were compared using Tukey's honestly significant difference (HSD; when homoscedastic) or Tamhane's T2 multiple comparison (when heteroscedastic) tests.

A categorical principal component analysis (CATPCA) with optimal scaling was used to explore the joint relationships between tomato accessions and their phenolic composition and antioxidant capacity. The number of dimensions to keep for data analysis was assessed by the respective eigenvalues (which should be greater than one), the Cronbach's alpha (that must be positive) and the total percentage of variance (that should be as higher as possible) explained by the selected data. The number of plotted dimensions (two) was chosen in order to allow meaningful interpretations.

3. Results and discussion

3.1. Phenolic composition

Data related to the phenolic compounds identification in the ripen fruits of table tomato accessions from the BPGV collection described in Table 1 are presented in Table 2, namely retention time, λ_{max} in the UV–Vis region, pseudomolecular ion, ions of major fragments in MS^2 and tentative identification. Fifteen phenolic compounds were identified, including 11 phenolic acids and 4 flavonoids. A similar phenolic profile was previously reported in hydromethanolic extracts of four different Portuguese tomato farmers' varieties (Barros et al., 2012), so that the identification made in this study was carried out taking into account all the characteristics of identification previously performed. As illustrative example, the HPLC phenolic profile of BPGV 11803 is presented in Fig. 1.

As shown in Table 3, the phenolic profile was very similar among all studied tomato accessions. However, the phenolic compounds content resulting from the sum of all the quantified metabolites ranged from 852 ± 5 μg/g extract in BPGV 12437 to 3935 ± 62 μg/g extract in BPGV16388 (Supplementary Fig. 1). These significant differences in the content of grouped phenolic compounds can be attributed to factors intrinsic to the germplasm itself (*i.e.*, genetic variability) (Siracusa et al., 2018). The levels of these secondary metabolites can also be affected by environmental and agronomic conditions, as well as treatments made during the fruits handling at the post-harvest stage (Asensio, Sanvicente, Mallor, & Menal-Puey, 2019; Coyago-Cruz et al., 2018). Nevertheless, as the analysed table tomato accessions were regenerated under the same edaphoclimatic conditions and cultivation techniques in the experimental fields of BPGV, possible variations caused by agroecological factors were not considered as discriminating factors.

Phenolic acids were found in higher quantity than flavonoids (or flavonols, since they were identified as quercetin and kaempferol derivatives in the conjugated form bound to sugar molecules). BPGV 16388 was also the accession in which the greatest amount of phenolic acids was quantified (3619 ± 53 μg/g extract), having been detected compounds such as caffeic acid, *p*-coumaric acid and compounds derived from these acids, the majority being *p*-coumaric acid bound to a hexose (1711 μg/g extract), followed by caffeic acid also bound to a hexose (816 μg/g extract) and finally 5-*O*-caffeoylquinic acid (376 μg/g extract) (Table 3). The accessions BPGV 11803, BPGV 11098 and BPGV 12506 also had high levels of phenolic acids (1 g of extract contained 2272 ± 24 μg, 1847 ± 29 μg and 1786 ± 28 μg of this class of compounds, respectively). In turn, BPGV 11098 was richer in flavonoids (365 ± 4 μg/g extract), particularly in quercetin-3-*O*-rutinoside (301 ± 3 μg/g extract), kaempferol-3-*O*-rutinoside (42.1 ± 0.6 μg/g extract) and quercetin-pentosyl-rutinoside (21.9 ± 0.3 μg/g extract). BPGV 16388 also had a considerable content of flavonoids (315 ± 9 μg/g extract), along with BPGV 11350; 322 ± 4 μg/g extract). According to Moco et al. (2007), flavonoids and their derivatives are typically found in the epidermal tissues of tomatoes, while phenolic acids can be detected as relatively high signals in all tissues of this fruit.

In a previouse study, Barros et al. (2012) also reported the group of phenolic acids (including *p*-coumaric acid derivatives and 4-*O*-caffeoylquinic acid) as the predominant group of molecules in four table tomato farmers' varieties grown in North-eastern Portugal homegardens. Quercetin-pentosyl-rutinoside was identified as the major flavonoid. Later, Pinela et al. (2016) described that quercetin-3-*O*-rutinoside (rutin) predominates over quercetin-pentosyl-rutinoside in the round tomato farmers' variety, in accordance to our results. In other works, Georgé et al. (2011) described caffeic acid derivatives as the

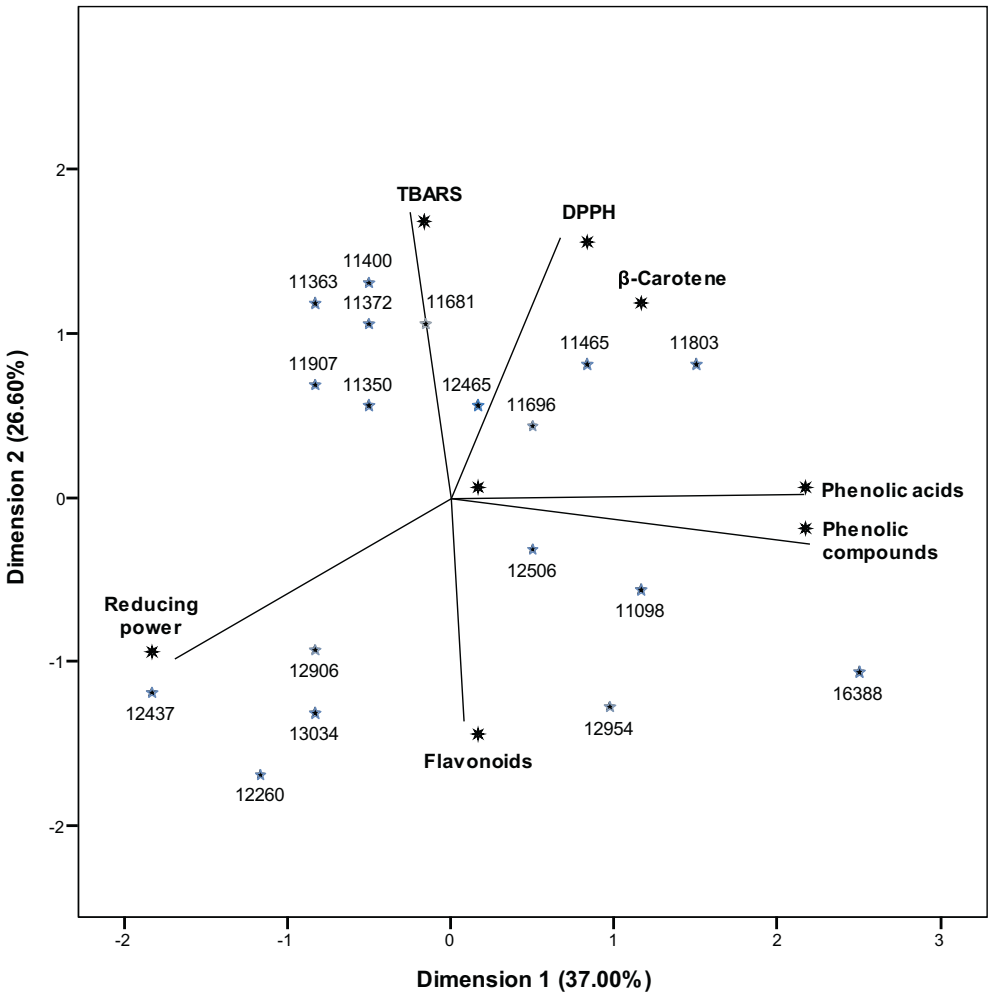

Fig. 2. Biplot of object scores (studied table tomato accessions) and component loadings (groups of phenolic compounds and antioxidant properties; indicated by vectors) from CATPCA. The first two dimensions (first: Cronbach's α, 0.734; eigenvalue, 2.590; second: Cronbach's α, 0.586; eigenvalue, 1.862) account for most of the variance.

main hydroxycinnamates in red and yellow tomatoes cultivated in Bellegarde, Southern France, while rutin and naringenin (a flavanone not identified in our samples) were identified as the main flavonoids. García-Valverde, Navarro-González, García-Alonso, and Periago (2013) identified chlorogenic acid (ester of caffeic and quinic acids, which isomers are 4-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid) and rutin as the most abundant phenolic compounds in Spanish tomato varieties for industrial processing and fresh consumption. The authors reported a variation in the amounts of all individual compounds as a function of the variety, and correlated the total phenolic content with hydrophilic antioxidant capacity. Vallverdú-Queralt et al. (2011) also concluded that phenolic and hydroxycinnamic acids and flavonoids, as well as hydrophilic antioxidant capacity can be used as chemotaxonomic markers to distinguish between tomatoes according to variety.

As presented in Table 1, some of the studied table tomato accessions have the same local name, which is generally related with locally valuable unique morphological or useful features. This is the case of BPGV 11098 and BPGV 11400, as well as accessions 11363, 12260, 12506, 12954, and 13034. Although they are locally known by the same vernacular name, each accession originates from different regions of Portugal. Actually, this crop has been grown for centuries by local farmers, who carried out distinct selections and contributed to the development of ecotypes adapted to the agroclimatic conditions and consumption preferences of their regions. This practice has led to the emergence of a great diversity of tomato landraces in Portugal, but also in other Mediterranean countries, whose local name was given by farmers (Cebolla-Cornejo, Roselló, & Nuez, 2013; Corrado, Caramante, Piffanelli, & Rao, 2014).

This study showed that accessions with similar local names and morphological features resulting from a different farmers' selection and diverse agroecological environments may present a different content of phenolic compounds, even when cultivated under the same conditions. For example, regarding germplasm of table tomato accessions widely known as “coração-de-boi” (literally meaning ox-heart tomato), it is possible to distinguish two groups relative to phenolic compounds: one consisting of BPGV 12506 and BPGV 12954, respectively from Santarém and Aveiro (with ~1913 μg/g extract); and another formed by the accessions BPGV 12260, BPGV 11363 and BPGV 13034 originating from Bragança, Santarém and Guarda, respectively (which contained 1221–1336 μg/g extract).

3.2. Antioxidant capacity

The results of the antioxidant capacity of the tomato accessions described in Table 1 are given in Table 4. Since there is no single method capable of evaluating all mechanisms of protection against oxidation, four *in vitro* assays were carried out to measure the reducing power, the ability to scavenge free radicals, and to inhibit lipid peroxidation phenomena. BPGV 11465, BPGV 11681 and BPGV 16388 stood out for their reducing power, with EC_{50} values of

0.54 ± 0.01 mg/mL, 0.73 ± 0.01 mg/mL, and 0.77 ± 0.03 mg/mL, respectively. Accession BPGV 16388 was also the one that had the highest content of phenolic compounds (particularly phenolic acids). Thereby, three accessions (BPGV 12437, BPGV 12906 and BPGV 12260) had a lower reducing capacity, translated by higher EC_{50} values (between 3.01 and 3.66 mg/mL). Even though, two of these samples (BPGV 12437 and BPGV 12260) were effective in scavenging DPPH free radicals (with EC_{50} values of 3.70 ± 0.03 mg/mL and 5.05 ± 0.09 mg/mL), and also in protecting β-carotene from free radicals generated from linoleic acid (EC_{50} value: 0.26 ± 0.01 mg/mL), or in inhibiting the formation of TBARS (EC_{50} value: 0.66 ± 0.04 mg/mL) generated from the *ex vivo* decomposition of lipid peroxidation products, respectively. Despite the low levels of phenolic compounds in BPGV 12437, this accession was distinguished by the quercetin-pentosyl-rutinoside content.

Extracts of BPGV 12954 and BPGV 12906 (both from Aveiro region) also displayed interesting TBARS inhibition capacity (Table 4). This means that these extracts have the ability to inhibit the formation of malondialdehyde, a reactive aldehyde produced by lipid peroxidation of the polyunsaturated fatty acids from the porcine brain cell membranes. This end product forms adducts with two thiobarbituric acid molecules to produce a pink colour species that absorbs at 532–535 nm (Dasgupta, Klein, Dasgupta, & Klein, 2014). The highest EC_{50} values of this assay were attributed to BPGV 11696 (2.5 ± 0.1 mg/mL) and BPGV 11400 (2.7 ± 0.1 mg/mL), both originating in the Santarem region, and BPGV 11350 (2.34 ± 0.08 mg/mL) from a region within Lisbon District. Among these, BPGV 11400 and BPGV 11350 also had a low β-carotene bleaching inhibition capacity (with EC_{50} values of 0.59 ± 0.01 mg/mL and 0.51 ± 0.03 mg/mL, respectively), together with BPGV 11803 and BPGV 11907, both from Portalegre (with EC_{50} values between 0.51 and 0.53 mg/mL). In this assay, β-carotene undergoes discoloration in the absence of antioxidants, which results in a reduction in the absorbance of the test solution with increasing reaction time. The presence of antioxidants hinders the extent of bleaching by neutralizing the linoleic hydroperoxyl radicals formed in the reaction emulsion (Kulisic, Radonic, Katalinic, & Milos, 2004).

In general, the obtained tomato extracts displayed a comparable TBARS formation inhibition and reducing capacities, a better β-carotene bleaching inhibition capacity, and a lower DPPH free radical scavenging activity compared to methanolic extracts obtained from tomato farmers' varieties in Miranda do Douro (North-eastern Portugal) homegardens (Pinela et al., 2012). Among these varieties, the so-called round tomato had the most powerful antioxidant activity (EC_{50} values ≤1.63 mg/mL) and phenolic content. In other study, George et al. (2004) measured the antioxidant capacity of 12 tomato genotypes grown in New Delhi, India, and verified that hexane fractions containing lycopene had higher antioxidant capacity than methanol fractions containing phenolics. Tomatoes are actually reservoirs of other antioxidant molecules than phenolic compounds, such as carotenoids, ascorbic acid and tocopherols, which can interact synergistically and enhance the antioxidant defence system (George et al., 2004; Pinela et al., 2012; Pinela, Oliveira, & Ferreira, 2016). The characterized tomato accessions can thus be seen as valuable genotypes, not only to improve the status of dietary antioxidants in the human diet, but also to increase nutritional value through germplasm enhancement programmes.

3.3. Selection of promising tomato germplasm

A CATPCA was performed to identify the most promising table tomato germplasm to be used in breeding programmes, considering the phenolic composition and antioxidant capacity characteristics. The biplot of Fig. 2 illustrates the joint relationships between the tomato accessions described in Table 1 (object scores) and their phenolic composition and antioxidant properties (component loadings). The first two dimensions (first: Cronbach's α, 0.734; eigenvalue, 2.590; second: Cronbach's α, 0.586; eigenvalue, 1.862) account for most of the total variance (37.00% and 26.60%, respectively) of the considered variables. The first dimension was effective in separating tomato accessions based on their composition in grouped phenolic compounds, grouped phenolic acids and reducing power. The second dimension, on the other hand, was particularly effective in separating accessions that differ in their TBARS formation inhibition capacity, DPPH free radical scavenging activity, and content of grouped flavonoids. Therefore, it was possible to observe that BPGV 16388 stands out for the phenolic content and reducing power, followed by BPGV 11098. The BPGV 11803 also had high levels of phenolic compounds but had a lower antioxidant capacity *via* β-carotene bleaching inhibition and DPPH free radical scavenging activity. Another interesting accession was BPGV 12954, which had interesting antioxidant properties and relatively high levels of phenolic compounds. Moreover, BPGV 12260 was also effective in scavenging DPPH free radicals and inhibiting the formation of TBARS, but the phenolic content was lower.

4. Conclusion

Among the 18 characterized table tomato accessions, BPGV 16388 stood out for its phenolic composition and antioxidant capacity, followed by BPGV 11098. Accession BPGV 12954 also had interesting antioxidant properties and relatively high levels of phenolic compounds. The obtained results are of interest to the management of the *ex-situ* collection, for their utilization in breeding programmes, and for their direct use in local and regional markets, considering high quality standards for fresh consumption. In addition, the characterized germplasm was a good model to evaluate the phenolic profile variation in tomato landraces. In further studies it will be interesting to associate the phenolic profile of these tomato accessions with variations at specific genomic regions in order to establish new criteria for distinctiveness and protection.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.108545.

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